COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled TREATMENT AND ANALYSIS OF PROLIFERATIVE DISORDERS, the specification of which:

[] is attached hereto.
[X] was filed on June 2, 2000 as Application Serial No. 09/586,235.
[] was described and claimed in PCT International Application No. _____ filed on _____ and as amended under PCT Article 19 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim the benefit under Title 35, United States Code, §119(e)(1) of any United States provisional application(s) listed below:

U.S. Serial No.	Filing Date	Status
60/137,365	June 3, 1999	Pending

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	HASAN ET AL.
Serial No.	:	09/586,235
For	:	INTEGRATED PHOTODYNAMIC AND DIFFERENTIATION THERAPY
Filed	:	June 2, 2000
Examiner	:	Travers
Art Unit	:	1614

745 Fifth Avenue
New York, NY 10151

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on August 27, 2001.

THOMAS J. KOWALSKI, REG. NO. 32,147
Name of Applicant, Assignee or Registered Representative

Signature
August 27, 2001
Date of Signature

DECLARATION UNDER 37 C.F.R. § 1.132

I declare as follows:

1. I am an inventor of U.S. patent application No. 09/586,235 ("the present application"), and am familiar with the present application, and its prosecution. My *curricula vitae* is attached under Tab A. I respectfully submit that I am qualified to speak and render opinions as to the disclosure in the present application and the state of the art, as I am considered to be an expert in the field and have familiarity with the present application and its prosecution. Furthermore, I performed, supervised or controlled any experimental work reported herein, in the ordinary course of business.

2. I am familiar with the Office Action dated February 27, 2001, issued by the United States Patent and Trademark Office in connection with the present application and make this Declaration in response thereto. I will address the following issues, which are presently in question by the Examiner:

- The inventive anticancer methods do not entail undue experimentation when carried out *in vivo*. Photodynamic therapy used in combination with cellular differentiation factors can be practiced with specificity and potency by one of skill in the art without undue experimentation in view of the knowledge in the art and the teachings in the present application.

3. The present invention relates to methods of inhibiting unwanted cellular proliferation using photodynamic therapy (PDT) in combination with a cellular differentiation factor. I have conducted experiments in accordance with the teaching in the specification, which demonstrates the successful use of the claimed methods *in vivo*. Using the claimed methods in vivo, one of skill in the art can attain adequate specificity and potency without the need for extensive experimentation.

4. Tumor-bearing animals were exposed to differentiation therapy (DT) using all trans retinoic acid (ATRA), ALA-PDT, or a DT/PDT combination, where a pretreatment with ATRA was followed by ALA-PDT. First, subcutaneous tumors were induced by injection of 100,000 EMT6 cancer cells. Tumor-bearing animals were then treated with a subcutaneous injection of 10 mg/kg body weight of ATRA, or with solvent as control. After 3 days, the animals received 100 mg/kg body weight of aminolevulinic acid and after 4 hours an irradiation of 100 J/cm^2 red (635 nm) light at a fluence rate of 200 mW/cm^2.

Treatment	Group 1	Group 2	Group 3	Group 4
Tumor implantation	+	+	+	+
ATRA	-	-	+	+
PDT	-	+	-	+

After additional 3 days, the animals were euthanized, the tumors excised and weighed for assessment of treatment efficacy.

4. The combination regimen of DT/PDT showed a marked enhancement of tumor reduction compared to PDT or ATRA alone. This is illustrated in Figure 1a, where tumor weight was recorded. Note that the lowest tumor weights were recorded with the combined regimen. Figure 1b illustrates this effect by showing tumors that were treated with PDT alone or in combination with ATRA, and an untreated control tumor. These results demonstrate the potency and specificity of a DT/PDT combination for the treatment of malignancies *in vivo*.

5. I carried out additional studies to evaluate the long-term therapeutic responses to DT/PDT. The combination of androgen treatment with ALA-based PDT was superior over either of the monotherapies. The effects of androgen therapy combined with PDT on LNCaP prostate cancer cells *in vitro* were studied using colony formation ability as a measurable endpoint. LNCaP cells were induced by DT using 0.1 μM of the synthetic androgen R1881 for 72 hours. Then, these cells and vehicle-treated control cells were treated with PDT using 0.3mM ALA and 4 hours later incremental irradiations of laser light. After the light exposure the cells were detached by trypsinization and diluted aliquots were replated on 100mm dishes to assess longterm survival of individual cells. This type of assay is called a colony formation assay. It is based on the fact that each single cancer cell plated at low density can give rise to one clonal colony of cells. After 13 days, the supernatants were removed and the colonies (clones) on the plates fixed, stained and counted. These clonogenic survival data are indicative of the long-term efficacy and applicability of DT/PDT combination regimens. These data also support the 24-hour survival assay (MTT assay) that have demonstrated the short-term efficacy of combination protocols.

6. The effect of a combination of differentiation therapy (DT; using the androgen analogue R1881) and ALA-based PDT is shown in Figure 2. PDT alone achieves minimal killing,

while the cells that were exposed to androgen-induced differentiation followed by PDT undergo a steep decline in survival following two PDT doses.

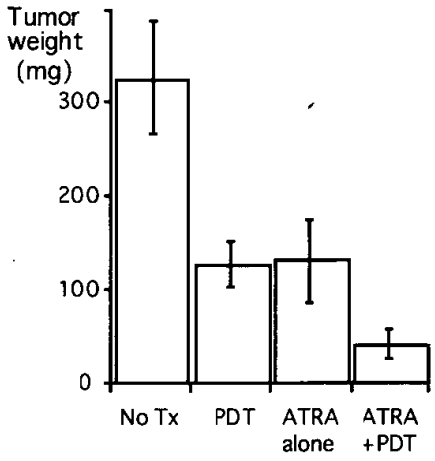

Figure 1a. ***Combination of DT and PDT in vivo.*** Mice carrying subcutaneous tumors were exposed to vehicle or DT using all trans retinoic acid (ATRA). After 72 hours 100 mg/kg ALA was injected intraperitoneally and 4 hours later the tumors were exposed to 100 J/cm^2 of 635 nm radiation at a fluence rate of 200 mW/cm^2. After another 3 days the mice were euthanized and the tumors excised and weighed. Mean values and standard deviations of tumor weights showed that the combined effect of ATRA and PDT was greater than either ATRA or PDT alone.

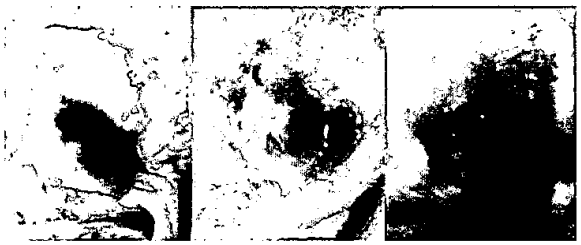

Figure 1b. ***Combination of DT and PDT in vivo.*** Three tumors are shown that are representative for the groups receiving: no treatment (left), PDT alone (middle), and the combination of ATRA and PDT (right). The untreated tumor and the tumor treated with PDT alone present a similar appearance with central necrosis. The combination protocol resulted in a crust-covered cutaneous defect with apparent tumor regression.

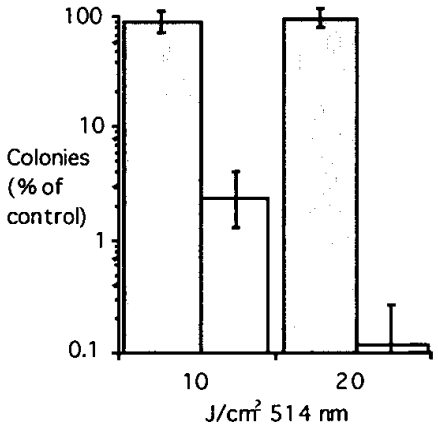

Figure 2. ***Longterm survival of LNCaP cells after PDT or DT+PDT.*** LNCaP cells were treated with either vehicle (grey bars) or 10-7M R1881 (white bars) for 96 hours and then incubated with 0.3 mM ALA for 4 hours. After exposure to 10 or 20 J/cm2 of 514 nm light delivered by an argon ion laser, cells were trypsinized and aliquots plated onto 10 cm dishes. After 14 days, the colonies were stained and counted. Survival was expressed as percent of unirradiated controls. While survival of cells which received PDT only was slightly reduced, cells pre-exposed to androgen treatment showed a large, dose-dependent reduction of long-term survival by subsequent PDT. This difference was statistically significant at p=0.005 level.

4. The Examiner questions whether the inventive methods will be selective for cells undergoing unwanted proliferation in a subject. Combination DT/PDT actually has advantages over conventional therapies in this regard. The differentiation factor will selectively target proliferating cells. The photosensitizer will also selectively target proliferating cells and as a result, the proliferating cells accumulate substantially greater amounts of photosensitizer. Third, it is well-known in the art that activating light can be targeted directly to the proliferating cells, and delivered in amounts specific to the threshold level of photosensitizer that has accumulated only in those cells. Thus, DT/PDT is actually more selective than conventional techniques.

5. There are several paradigms of differentiation therapy that further illustrate its selectivity for proliferating cells. The treatment with systemic retinoids of the hyperproliferative skin disease psoriasis leads to a profound therapeutic effect on lesional skin within days, while normal skin is basically unaltered. Similarly, in differentiation therapy of acute promyelocytic leukemia, diseased cells are differentiated by systemic retinoic acid, while normal white blood cells are not affected. This virtual absence of severe side effects in normal tissues have made retinoids an important part of many combination regimens in cancer therapy.

6. Therefore, all evidence — the teachings in the present application, and the data herein that is consistent with the application teachings, and the knowledge in the art, *e.g.*, as evinced by herein cited documents — indicates that it is feasible for the inventive methods to combine PDT with a variety of differentiation factors known in the art and for the inventive methods to be successfully employed to inhibit unwanted cellular proliferation *in vivo* without undue experimentation. Consistent with the teachings in the specification, I have shown that (1) DT *in vitro* makes a variety of animal-derived and human cells more susceptible PDT, (2) DT *in vitro* enhances PDT effects on short-term and longterm survival of cancer cells, (3) DT *in vivo* results in increased PS formation in malignant tumors, and (4) DT enhances efficacy of PDT of tumors *in vivo*.

7. The teachings in the application, as discussed above, are quite detailed, the level of skill in the art is high, and there is a body of literature showing that no undue experimentation is needed to practice the claimed invention. Reconsideration and withdrawal of the Section 112 rejections of the application is respectfully requested.

8. I further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

Dated August 24, 2001 By: _____
Bernard Ortel